

WHAT I CLAIM IS:

1. An insulin regulator construct, comprising:
  - a) a glucose response element (GIRE) of a liver-pyruvate (L-PK) gene promoter; and
  - b) an insulin-sensitive element of an insulin-like growth factor binding protein-1 (IGFBP-1) basal promoter.
2. The insulin regulator construct of Claim 1, wherein:

said glucose response element comprises a hepatic nuclear factor-4 (HNF-4) binding site and a glucose responsive site.
3. The insulin regulator construct of Claim 2, further comprising:

a plurality of said glucose response elements.
4. The insulin regulator construct of Claim 2, wherein:

the sequence of said HNF-4 binding site and said glucose responsive site is in a native orientation.
5. The insulin regulator construct of Claim 2, wherein:

the sequence of said HNF-4 binding site and said glucose responsive site is reversed from a native orientation.

6. The insulin regulator construct of Claim 1, wherein:  
said glucose response element is inserted upstream of said insulin-sensitive element in an insulin-like growth factor binding protein-1 (IGFBP-1) basal promoter.
7. The insulin regulator construct of Claim 1, wherein:  
said glucose response element comprises a nucleotide sequence set forth in SEQ ID NO.: 1.
8. The insulin regulator construct of Claim 1, wherein:  
said insulin-sensitive element comprises a nucleotide sequence set forth in SEQ ID NO.: 2.
9. An insulin regulator construct, comprising:  
a nucleotide sequence set forth in one of SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, and SEQ ID NO.: 6.
10. The insulin regulator construct of Claim 1, which is not stimulated by exposure to lactate or fructose.

11. The insulin regulator construct of Claim 1, which is stimulated by exposure to glucose and inhibited by exposure to insulin.
12. A vector comprising the construct of Claim 1.
13. An adenoviral vector comprising the construct of Claim 1.
14. A transgene comprising the construct of Claim 1.
15. A pharmaceutical composition comprising the construct of Claim 1 and a pharmaceutically acceptable carrier or diluent.
16. A pharmaceutically acceptable derivative of the construct of Claim 1.
17. A method of treating or preventing diabetic conditions in a subject by administering an effective amount of the construct of Claim 1.
18. A method of regulating insulin production in a subject by administering an effective amount of the construct of Claim 1.

19. A method of modulating hyperglycemia, while avoiding severe hypoglycemia, in a subject by administering an effective amount of the construct of Claim 1.
20. A method of increasing fat catabolism in a subject by administering an effective amount of the construct of Claim 1.
21. A method of reducing protein catabolism in a subject by administering an effective amount of the construct of Claim 1.

## REFERENCES

1. Eisenbarth GS. Type I diabetes mellitus: A chronic autoimmune disease. N Engl J Med 1986; 314:1360-1368.
2. Falqui L, Martinenghi S, Severini GM, et al. Reversal of diabetes in mice by implantation of human fibroblasts genetically engineered to release mature human insulin. Human Gene Therapy 1999; 10:1753-1762.
3. Muzzin P, Eisensmith RC, Copeland KC, Woo SLC. Hepatic insulin gene expression as treatment for Type 1 diabetes mellitus in rats. Mol Endo 1997; 11:833-837.
4. Gros L, Riu E, Montoliu L, Ontiveros M, Lebrigand L, Bosch F. Insulin production by engineered muscle cells. Human Gene Therapy 1999; 10:1207-1217.
5. Short DK, Okada S, Yamauchi K, Pessin JE. Adenovirus-mediated transfer of a modified human proinsulin gene reverses hyperglycemia in diabetic mice. American Journal of Physiology 1998; 275:E748-E756.
6. Rivera VM, Wang W, Wardwell S, et al. Regulation of protein secretion through controlled aggregation in the endoplasmic reticulum. Science 2000; 287:826-830.
7. Selden RF, Skoskiewicz MJ, Russell PS, Goodman HM. Regulation of insulin-gene expression. N Engl J Med 1987; 317:1067-1076.
8. Kolodka TM, Finegold M, Moss L, Woo SLC. Gene therapy for diabetes mellitus in rats by hepatic expression of insulin. Proc Natl Acad Sci USA 1995; 92:3293-3297.
9. Tuch BE, Tabiin MT, Casamento FM, Simpson AM, Marshall GM. Transplantation of genetically engineered insulin-producing hepatocytes

- into immunoincompetent mice. Transplantation Proceedings 1998; 30:473.
10. Valera A, Fillat C, Costa C, et al. Regulated expression of human insulin in the liver of transgenic mice corrects diabetic alterations. FASEB J 1994; 8:440-447.
  11. Kaneda Y, Iwai K, Uchida T. Introduction and expression of the human insulin gene in adult rat liver. Journal of Biological Chemistry 1989; 264:12126-12129.
  12. Yamaguchi M, Kuzume M, Matusumoto T, et al. Insulin gene transfer compensates pancreatic  $\alpha$ -cell function in diabetic rats. Transplantation Proceedings 1998; 30:2913.
  13. Sugiyama A, Hattori S, Tanaka S, et al. Defective adenoassociated viral-mediated transfection of insulin gene by direct injection into liver parenchyma decreases blood glucose of diabetic mice. Hormone and Metabolic Research 1997; 29:599-603.
  14. Abai A, Hobart P, Barnhart KM. Insulin Delivery with Plasmid DNA. Human Gene Therapy 1999; 10:2637-2649.
  15. Lu D, Tamemoto H, Shibata H, Saito I, Takeuchi T. Regulatable production of insulin from primary-cultured hepatocytes: insulin production is up-regulated by glucagon and cAMP and down-regulated by insulin. Gene Therapy 1998; 5:888-895.
  16. Gros L, Montoliu L, Riu E, Lebrigand L, Bosch F. Regulated production of mature insulin by non-b-cells. Human Gene Therapy 1997; 8:2249-2259.
  17. Wanke IE, Wong NC. Specific problems facing gene therapy for insulin-dependent diabetes mellitus: glucose-regulated insulin secretion from hepatocytes. Proceeding of the Western Pharmacology Society 1997; 40:131-133.

18. Simpson AM, Marshall GM, Tuch BE, et al. Gene therapy of diabetes: glucose-stimulated insulin secretion in a human hepatoma cell line (HEP G2ins/g). Gene Therapy 1997; 4:1202-1215.
19. Powell DR, Suwanichkul A, Cubbage M, Lee PDK. Regulation of insulin-like growth factor binding protein-1 (IGFBP-1) protein levels, mRNA levels and promoter activity by insulin (IN) and IGF-1 in HepG2. Endo Society 1990:280A.
20. Powell DR, Suwanichkul A, Cubbage ML, DePaolis LA, Snuggs MB, Lee PDK. Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1. Journal of Biological Chemistry 1991; 266:18868-18876.
21. Powell DR, Suwanichkul A. HNF1 activates transcription of the human gene for insulin-like growth factor binding protein-1. DNA and Cell Biology 1993; 12:283-289.
22. Suwanichkul A, Cubbage ML, Powell DR. The promoter of the human gene for insulin-like growth factor binding protein-1. Basal promoter activity in HEP G2 cells depends upon liver factor B1. Journal of Biological Chemistry 1990; 265:21185-21193.
23. Suwanichkul A, DePaolis LA, Lee PDK, Powell DR. Identification of a promoter element which participates in cAMP-stimulated expression of human insulin-like growth factor-binding protein-1. Journal of Biological Chemistry 1993; 268:9730-9736.
24. Suwanichkul A, Morris SL, Powell DR. Identification of an insulin-responsive element in the promoter of the human gene for insulin-like growth factor binding protein-1. Journal of Biological Chemistry 1993; 268:17063-17068.
25. Suwanichkul A, Allander SV, Morris SL, Powell DR. Glucocorticoids and insulin regulate expression of the human gene for insulin-like growth

- factor-binding protein-1 through proximal promoter elements. Journal of Biological Chemistry 1994; 269:30835-30841.
26. Hughes SD, Johnson JH, Quaade C, Newgard CB. Engineering of glucose-stimulated insulin secretion and biosynthesis in non-islet cells. 1992; 89:688-692.
  27. Rencurel F, Waever G, Antoine B, et al. Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT 2) gene expression in liver. Biochemical Journal 1996; 314:903-909.
  28. Villafuerte BC, Goldstein S, Murphy LJ, Phillips LS. Nutrition and Somatomedin XXV. Regulation of insulin-like growth factor binding protein-1 in primary cultures of normal rat hepatocytes. Diabetes 1991; 40:837-841.
  29. Ooi GT, Tseng LY-H, Tran MQ, Rechler MM. Insulin rapidly decreases insulin-like growth factor-binding protein-1 gene transcription in streptozotocin-diabetic rats. Molecular Endocrinology 1992; 6:2219-2228.
  30. Pao C-I, Farmer PK, Begovic S, Goldstein S, Wu G-J, Phillips LS. Expression of hepatic insulin-like growth factor-I and insulin-like growth factor-binding protein-1 genes is transcriptionally regulated in streptozotocin-diabetic rats. Molecular Endocrinology 1992; 6:969-977.
  31. Suh D-S, Zhou Y, Ooi GT, Rechler MM. Dexamethasone stimulation of rat insulin-like growth factor binding protein-1 (IGFBP-1) promoter activity involves the interaction of multiple transcription factors. Progress in Growth Factor Research 1995; 6:131-140.
  32. Cuif M-H, Cognet M, Boquet D, Tremp G, Kahn A, Vaulont S. Elements responsible for hormonal control and tissue specificity of L-type pyruvate kinase gene expression in transgenic mice. Molecular and Cellular Biology 1992; 12:4852-4861.



33. Cognet M, Lone YC, Vaulont S, Kahn A, Marie J. Structure of the rat L-type pyruvate kinase gene. J Mol Biol 1987; 196:11-25.
34. Bergot M-O, Diaz-Guerra M-JM, Puzenat N, Raymondjean M, Kahn A. Cis-regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. Nucleic Acids Research 1992; 20:1871-1878.
35. Vaulont S, Munnich A, Decauz J-F, Kahn A. Transcriptional and post-transcriptional regulation of L-type pyruvate kinase gene expression in rat liver. Journal of Biological Chemistry 1986; 261:7621-7625.
36. Goswami R, Lacson R, Unterman T. Identification of insulin and glucocorticoid response sequences in the rat IGF binding protein-1 (IGFBP-1) promoter. Endocrine Society 1993; 1915B:529.
37. Shu D-S, Ooi GT, Lesniak MAS. Inhibition of IGFBP-1 gene expression by insulin and stimulation by dexamethasone, cyclic amp, and phorbol esters are mediated by different cis-acting elements in the rat IGFBP-1 promoter. Endocrine Society 1993; 1916B:529.
38. Bergot M-O, Diaz-Guerra M-JM, Puzenat N, Raymondjean M, Kahn A. Cis -regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. Nucleic Acids Res 1992; 20:1871-1878.
39. Smeekens SP, Chan SJ, Steiner DF. The biosynthesis and processing of neuroendocrine peptides: identification of proprotein convertases involved in intravesicular processing. Progress in Brain Research 1992; 92:235-246.
40. Groskreutz DJ, Sliwowski MX, Gorman CM. Genetically engineered proinsulin constitutively processed and secreted as mature, active insulin. Journal of Biological Chemistry 1994; 269:6241-6245.
41. Steiner DF, Smeekens SP, Ohagi S, Chan SJ. The New Enzymology of Precursor Processing Endoproteases. Journal of Biological Chemistry 1992; 267:23435-23438.

42. Simonson GD, Groskreutz DJ, Gorman CM, MacDonald MJ. Synthesis and processing of genetically modified human proinsulin by rat myoblast primary cultures. Human Gene Therapy 1996; 7:71-78.
43. Unger RH, Foster DW. Chapter 21. In: Wilson JD, Foster DW, Kronenberg HM, Williams RH, eds. Williams Textbook of Endocrinology. Vol. 9th. Philadelphia, London, Toronto, Montreal, Sydney: W.B Saunders Co., 1998:973-1059.
44. Robertson DG, Marino EM, Thule PM, Seneviratne CK, Murphy LJ. Insulin and glucocorticoids regulate IGFBP-1 expression via a common promoter region. Biochemical Biophysical Research Communication 1994; 200:226-232.
45. Goswami R, Lacson R, Yang E, Sam R, Unterman T. Functional analysis of glucocorticoid and insulin response sequences in the rat insulin-like growth factor-binding protein-1 promoter. Endocrinology 1994; 134:736-743.
46. Suh DS, Ooi GT, Rechler MM. Identification of cis -elements mediating the stimulation of rat insulin-like growth factor-binding protein-1 promoter activity by dexamethasone, cyclic adenosine 3',5'-monophosphate, and phorbol esters, and inhibition by insulin. Molecular Endocrinology 1994; 8:794-805.
47. Goldstein S, Sertich G, Levan KR, Phillips LS. Nutrition and somatomedin. XIX. Molecular regulation of insulin-like growth factor-I in streptozotocin-diabetic rats. Molecular Endocrinology 1988; 2:1093-1100.
48. Minematsu S, Watanabe M, Tsuchiya N, Amagaya S. Diurnal variations in blood chemical items in Sprague-Dawley rats. Experimental Animals 1995; 44:223-232.

49. Haughton CL, Dillehay DL, Phillips LS. Insulin replacement therapy for the rat model of streptozotocin-induced diabetes mellitus. Laboratory Animal Science 1999; 49:639-44.
50. Koopmans SJ, Sips HCM, Krans HMJ, Radder JK. Pulsatile intravenous insulin replacement in streptozotocin-diabetic rats is more efficient than continuous delivery: effects on glycaemic control, insulin-mediated glucose metabolism and lipolysis. Diabetologia 1996; 39:391-400.
51. Wang RN, Bouwens L, Kloeppel G. Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. Diabetologia 1994; 37:1088-1096.
52. Like AA, Guberski DL, Butler L. Influence of Environmental Viral Agents on Frequency and Tempo of Diabetes Mellitus in BB/Wor Rats. Diabetes 1991; 40:259-262.
53. Seglen PO. Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. Exp Cell Res 1973; 82:391-398.
54. Ginot F, Decaux J-F, Cognet M, et al. Transfection of hepatic genes into adult rat hepatocytes in primary culture and their tissue-specific expression. Eur J Biochem 1989; 180:289-294.
55. Baker A, Saltik M, Lehrmann H, et al. Polyethylenimine (PEI) is a simple, inexpensive and effective reagent for condensing and linking plasmid DNA to adenovirus for gene delivery. Gene Therapy 1997; 4:773-782.
56. Marriott D, Gillece-Castro B, Gorman CM. Prohormone convertase-1 will process prorelaxin, a member of the insulin family of hormones. Molecular Endocrinology 1992; 6:1441-1450.
57. Mittereder N, March KL, Trapnell BC. Evaluation of the concentration and bioactivity of adenovirus vectors for gene therapy. Journal of Virology 1996; 70:7498-7509.